Sigmaplot

~~* select the return~~

① Statistics

② Regression wizard

③ Sigmoidal ~~3. parameter~~

④ Logistic 4. parameter

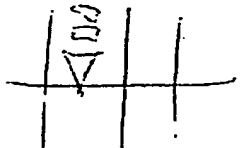
(if higher parameter does not work, use lower one (3))

⑤ Parameters: First empty
need in report ☑
create new graph ☑

↓

Finish.

* title on column



type

* folder list own name change

Rt click : Edit click

→ type new name

BEST AVAILABLE COPY

① IgG 1mg/ml 50ul 1hr
② Block 200ul 1% BSA
③ Enz anti-IgG 50ul
Substrate 50ul

50ul

(5)

IgG (4g dilute)

1:10 1:10 1:100 1:100 1:500 1:500 1:1000 1:1000

	1	2	3	4	5	6	7	8	9	10	11	12 (50ul)
A	Blank	IgG	1:10	1:100	1:100							1:1000
B		1:10	1:10									"
C		1:10	1:10									1:2000
D		1:10	1:10									"
E		1:10										1:4000
F		1:10										"
G												
H												

(5) (ml)
1 → 1000 ml
0.1 → 100
0.01 → 10
0.005 + 5ml

(4.995)

2ml + 2ml PBS

0.1% BSA-TBS

50ul

12 x 0.05ml (1ml) PBS

.05
.60

1:10 1ml (0.2ml 1.8ml) 0.1ml + 0.9ml PBS
1:100 1ml 0.2ml + 1.8ml
1:500 1ml 0.2ml + 0.8ml
1:1000 1ml 0.1ml (100) + 0.9ml

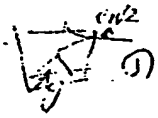
0.9ml

1:10 / 1:10
1:100
0.1 + 0.9
0.2 + 1.8

1ml = 1000ul
0.2ml = 200ul

	1	2	3	4	5	6	7	8	9	10	11	12
A	3kng											
B												
C												
D												
E												
F												
G												
H												

1 substrate - yellow



1) Antigen — 1hr to overnight in bicarbonate buffer of H.S.O (sticky)

2) Wash x add blocking reagent 1% BSA and PBS (phosphate buffered saline)
↳ 1 hr wait

3) Add Antibody (human serum)
↳ 1 hr wait

4) Wash

5) Add indirect enzyme conjugate Ab
↳ 1 hr wait

6) Wash → Add Substrate

Ag {
- 1:100 (bicarbonate) — 20 µl + 2 ml
- 1:500 (") — 200 µl + 0.8 ml
- 1:1000 (") — 200 µl of (1:100) + 1.8 ml bicarbonate
- 1:2000 (") — 0.05 ml (50 µl of 1:100) + 1.0 ml bicarbonate

human IgG in bicarb

	1:100	1:100	1:500	1:500	1:1000	1:1000	1:2000	1:2000					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
A	Blank												1:100
B	PBS												
C													1:2000
D													
E													1:4000
F													1:2000
G						NOTH							NOTH
H													1:4000 ~~1:2000~~

Ag
- ✱ 1:100 → .1 v 10.0
  - .01 → 1 ml (20 µl + 2 ml) — 12 x 0.05 ml = .60 ml
- ✱ 1:500 — 1:5 dil = 1:500 — 0.1 ml v 0.4; 0.2 ml v 0.8
- 1:1000 dil'n — 0.1 ml + 0.9 ml (0.2 ml + 1.8)
- 1:2000 dil'n ⇒ 1:20 dil'n — 1 → 20 or .1 → 2.0
  - 0.1 ml → 2.0 ml
  - 0.05 ml → 1.0 ml

0.05 → 1.0 ml (1:20)

① Eliza : basic concept.

Ag (thyroglobin) Anti Ab

Block + Pt's Serum (1:50 dilution
(1% Bovine serum albumin) (Anti Ab to thyroglobin)
{ 1ml → 50 ml
0.1ml → 5 ml
0.01ml → 0.5ml
(10 μl → 500 μl)

Ig (thyroglobin)
(Ag)

(goat anti human IgG
AP: Alkaline phosphatase
(human)

(2)

IgG

1000 µg/ml in PBS

1:100

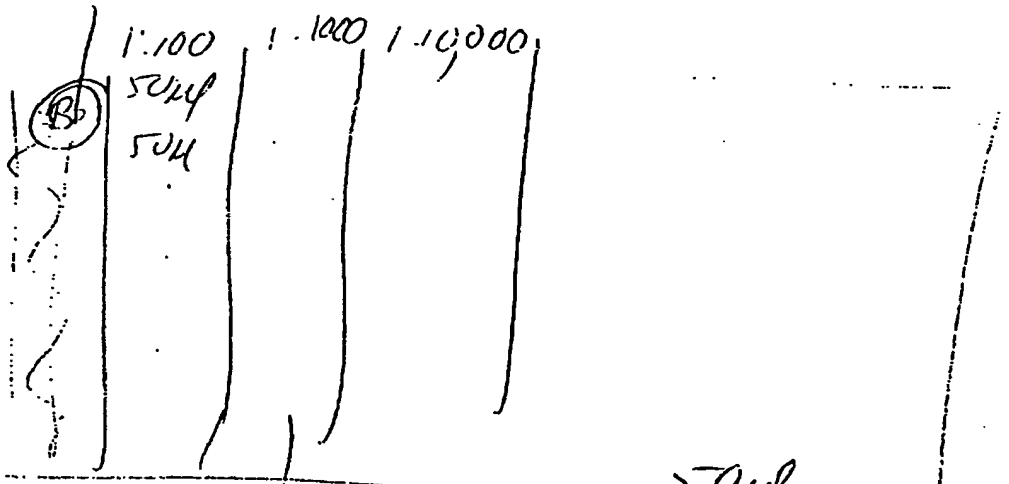
1:1000

1:10,000

IgG
Alk phosph anti human
IgG
substr

= Bicarbonate buffer.

Bicarb 50µl



Load on 1 hr RT
Wash 4x = PBS-tween 20 0.2% 200µl
Blocke with 1% BSA Hr / hr (200µl)
Dump
Add ~~conte~~ AP anti-human IgG (50µl) 1hr in 0.1% BSA
Wash 4x PBS-Tween 20 (200µl)
Add substrate (50µl) 30min 37°C
Add stopping reagent (optional)
Read OD

1ml : 199 ml PBS

① anti-CD Ab ( ) mg/ml
→ dilute 1:200 in PBS [No BSA, No Serum],
place 30 ul in each well for stimulation.

② Incubate 1 hr at 37°C or
Overnight at 4°C (refrigerator)

③ Wash cells 2 x with 200 ul PBS - tap out on paper towel (should be sterile)

~~④~~ * No stim cells at least 1/ away from stim

Stim		
●	○	○
●	○	○
●	○	○
●	○	○

④ 200,000 cells/well in 200 ul
(= 1 x 10⁶/ml)

⑤ for flow, Centrifuge in regular tubes, put supernatant into eppendorf.

⑥ collect S/N at 24 hrs, Centrifuge in microfuge and place in a fresh tube

⑦ Assay by ELISA immediately or
freeze S/N ≤ -20°C

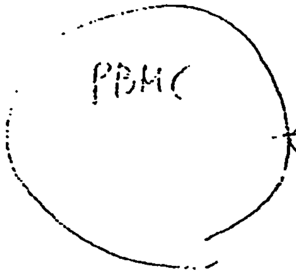
if multiple ELISA; Aliquot S/N.

— Read ELISA protocol ahead of time
How much sample do you need?

IL-10 1:10 → 100 ul

~~PHA~~ (2 µg) µg/ml + αCD3] compare
− αCD3]

(*) R & D Systems
DO NOT USE BIOSOURCE
(BSE ?)



Stim
Anti CD3

IL-2 ⇓ (IFNγ) Th1
IL-10 ⇑ ([illegible])

Rx (10 ml women
10 preg women

αCD Ab 2 ng/ml (1 mg/ml)

anti CD3 10 µg/ml

in PBS (NO BSA NO SERUM)

(1) dilute 1:200, place 30 µl in each well for Stimulation

(2) incubate (1 hr at 37°C) or overnight at 4°C (refrig.) (sterile)

(3) wash wells 2x with 200 µl PBS - tap out on paper towel.

* no stim wells at least 1 away from stim

(4) 200,000 cells/well in 200 µl = 1×10⁶/ml) → 24 hr incubate (37°C CO₂)

(5a) for flow, c-fuge in regular tubes, put S/N into eppendorf

(5) collect S/N at 24 hrs centrifuge in microfuge and place in a fresh tube. 5 min
→ collect the supernatant c Eppendorf pipette

(6) assay by ELISA immediately or

freeze S/N ≤ -20°C
if multiple ELISA's, aliquot S/N

- read ELISA protocol ahead of time
HOW MUCH SAMPLE DO YOU NEED?

TC-10

Coulter Epics (Turn On)

① Computer Power On → (wait 20 min)

② 맨 왼칸 뚜껑 (가운데 검은 box) - orange line 조심
~~[illegible]~~ · waste box check - ½ 이상이면 dump
· (2 white bottle - dry 해야
(2 Transparent bottle ⅔ 이하 해야
· Orange 읽어서 → (기록)

③ Panel → Select → start up click & okay click

④ 뚜껑 맨 밑칸 Run 누르 green blink 되면
open the door (옆의 긴 drawer)
→ Isoflud 채우고
button 2-3번 눌러 bubble 없는지 check

⑤ 화면에 Error message - click
Clear Error - click

⑥ Carrossal 에 3개 tube
① Water 1 ml 정도
② F-check : 10 drop
③ F-set : 10 drop

⑦ 뚜껑 [Run] 눌러 initialization orange 해제 안됨

⑧ Insert tube 화면 뜨면
okay click → 5-7분 wait

⑨ Flow-check 결과 :
Flow-Set "

HPCV = CV
MnFX = Mean CH
MnX = Peak CH copy 함

⑩ Protocol → Select.

< PCR 에 입력 >

Am or Listmode

region - create

color click (colorful 하게 [illegible])

⇓

File

↓

PCX File

box 크기 조절해서 PCR에 입력되는 숫자 사진을

저장합니다.

(左 mouse button 누른 채로 drag → left click 안됨)

⇓

PCX File

D:\ (하드 drive에 입력할 것)

C:\XL\DATA\CD56.PCX

(다른 protocol로 Data를 뽑아 넣을 것)

Listmode [Runtime protocol]

→ New protocol/panel

Shut down
① water
② ~~sol~~ bleach
③ water
④ water
} about 1 ml

Panel
→ Select
→ shut down
→ Run
(Manual drain)

tube 넣은 후 (take 8-10 min)
Run button → green → Push button
↳ it will be blinking

put the water 2X
black tube oil (양쪽 다 넣음)
in manual talk

green + blinking → I can open the door
(이때 안 깜빡이면 다시 누름)

→ take out test tube
→ black tube 넣음
→ 반드시 black tube 넣음

→ test tube 다시 넣음
Auto mode procedure ①. ②
put 2 tube water
carousel 에 넣음

→ [Auto] [Escape] → 종료에 누름

CD45 FITC / CD14 PE
CD3/CD4
CD3/CD8
CD5/CD19
CD3/CD23
CD56/CD16

Cytokines
IL-1
IL-2
IL-3
↓
IL-20

K562 Target

NK : Effector

50:1
50X more effector
target

100,000 target : 50:1
50 x 100,000 Lymphocytes

5×10^6

25 x 100,000
2.5×10^6

E:T 50:1 — 10%
E:T 25:1 — 5%
E:T 12:1 — 5%

2 hrs
after
measure
killing

Propidium iodide binds DNA

same K562 — 1000, 900
same — 100

10%

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